Version 19-08/24

User's Manual

Rat Kilham Virus IgG ELISA Kit

REF **DEIAJX006**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Rat anti-Kilham Rat Virus IgG ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for KRV major capsid protein (VP2) antigen in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

General Description

Three serogroups of parvoviruses affect rats: PV (including Kilham rat virus, KRV), RPV and H-1 (Toolans). Parvoviruses are typically linear, non-segmented ssDNA viruses with an average genome size of 5Kb, and target replicating tissue causing cell and tissue destruction. This explains why RV, in its active stage, will interfere with the formation of offspring, causing small litters, stillborns, or the resorption of litters.The viral capsid of a parvovirus is made up of two or three proteins, known as VP1-3, that form an icosahedral structure; VP2 is highly immunogenic.

KRV infection may be diagnosed by ELISA, measuring rapidly rising antibody titers (8-12 days after infection) to KRV antigen. Animals infected with KRV are not suitable for animal research; in addition to lower growth rates, KRV may predispose to secondary bacterial infection, cause infertility, and death in susceptible strains. Besides infecting animals, KRV may also contaminate cell lines, transplantable tumors and other biological products; these should be tested by rat antibody production (RAP), using ELISA to detect anti- KRV after immunization.

RecombiVirus ELISAs are 2nd generation immunoassays using highly purified, recombinant and antigenic proteins of animal viruses. The Creative Diagnostics Rat Anti-KRV IgG ELISA is designed with high sensitivity for the detection of antibodies during KRV infection.

Principles of Testing

The Rat Anti-KRV IgG ELISA kit is based on the binding of antiKRV IgG in samples to KRV antigen immobilized on the microwells, and anti- KRV IgG antibody is detected by anti- IgG- specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- KRV IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The presence of rat IgG antibody in samples is determined relative to rat anti-KRV IgG Controls.

Reagents And Materials Provided

1. Wash Solution Concentrate (100x): 10ml.
2. Sample Diluent Concentrate (20x): 10ml.
3. Anti-Rat IgG- HRP Conjugate Concentrate (100x): 0.15ml.
4. KRV Ag Microwell Strip Plate: 8-well strips (12).
5. Rat AntiKRV Sensitivity Control: 0.65 ml.

6. Rat AntiKRV Positive Control: 0.65 ml.
7. Low NSB Sample Diluent: 30 ml.
8. TMB Substrate: 12 ml.
9. Stop Solution: 12 ml.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml.
2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Rat IgG HRP Concentrate.
3. Stock bottle to store diluted Wash Solution: 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. Microwell plate reader at 450 nm wavelength and ELISA plate washer

Storage

2-8°C until the expiration date printed on the box label.

Specimen Collection And Preparation

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example:

Initial (1/5): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1/100): 10ul initial (1/5) + 190 ul LNSD

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

1. Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control to be assayed.
2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

3. Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

1. Wash Solution Concentrate (100x): Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at 4°C for long term and ambient temp. for short term.
2. Sample Diluent Concentrate (20x): Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
3. Anti-Rat IgG- HRP Conjugate Concentrate (100x): Peroxidase conjugated anti-rat IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

Assay Design

Review Calculation of Results and Limits of the Assay before proceeding:

Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the Sensitivity Control. This is usually 1/100 or greater dilution for rat sera with normal levels of IgG and IgM.

Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.

Run the Positive and Sensitivity Controls, which validate that the assay was performed to specifications: the Positive Control should give a high signal (>1.5 OD); the Sensitivity Control should give a low signal which can be used to discriminate at the Positive/Negative threshold.

1. 1 st Incubation [100ul – 60 min; 4 washes]

Add 100ul of calibrators, samples and controls each to predetermined wells.

Tap the plate gently to mix reagents and incubate for 60 minutes.

Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2 nd Incubation [100ul – 30 min; 5 washes]

Add 100ul of diluted Anti-Rat IgG HRP to each well.

Incubate for 30 minutes.

Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

Add 100ul of Stop Solution to each well.

Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

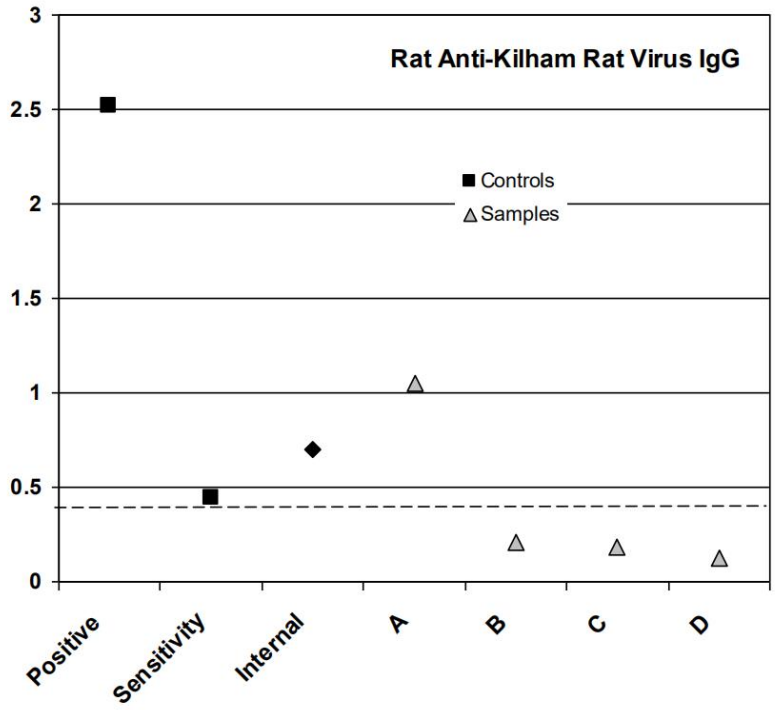
Interpretation Of Results

A. Antibody Activity Threshold Index

Compare Samples to Sensitivity Control or Internal Control

= Positive/Negative Cut-off.

Example:



Results

The sensitivity of the assay to detect anti-KRV IgG, from either natural infection or RAP (rat antibody production), is controlled so that the Sensitivity Control represents a threshold OD for most true positives in

rat serum diluted in the Low NSB Sample Diluent at 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Positive Control – clearly positive, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

Sensitivity Control – a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Internal Control – a true positive from an infected animal that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the Sensitivity Control for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (B, C, D) are negative: below the threshold; 1 sample (A) is positive: clearly above the threshold. The Sensitivity Control can be used to calculate a Threshold Index that numerically discriminates Positive/Negative, as follows:

Divide each Sample net OD by the Sensitivity Control net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

This calculation was used to represent Assay Precision.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Example:

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.195	**P** 2.345	0.90	**10.86**
2	0.189	**S** 0.374	0.88	**1.73**
3	0.137	**R** 1.080	0.63	**5.00**
4	0.155	**I** 0.582	0.72	**2.69**
5	0.073	**N** 0.779	0.34	**3.61**
6	0.149	**N** 0.990	0.69	**4.58**
7	0.090	**N** 0.773	0.42	**3.58**
8	0.121	**N** 0.993	0.56	**4.60**
9	0.149	**N** 2.357	0.69	**10.91**
10	0.122	**U** 0.225	0.56	1.04
Mean	0.138			
SD	0.039			
Mean +2 SD	**0.216**	= **Positive Index**		

Results

Experimental Samples are represented as follows:

P – Positive Control

S – Sensitivity Control

I – Internal Control; lab's threshold positive serum

U – Uninfected rat sample

R – Rat Antibody Production (RAP) sample represents injection of KRV antigen (same as used for plate coating) into rats; positive indicates presence of the KRV in the inoculum.

N – Naturally infected rat samples.

Notes:

The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a Positive Index (page 6), or by using known Internal Controls as discriminator for a Threshold Control (instead of the kit Sensitivity Control)

Precision

Samples and Controls were assayed in duplicate in 5 separate runs, to provide a measure of between-assay reproducibility.

The data are represented using the value of the Sensitivity Control in each assay to calculate a Threshold Index for each control and samples.

Sample	Ave OD	Threshold Index (mean)	Inter-assay %CV
Positive Control	2.33	7.83	**3.6**
Sensitivity Control	0.30	**1.00**	**0**
Internal Control	0.56	1.87	**4.1**
Natural Positive	0.88	2.94	**6.8**
RAP Positive	1.03	3.47	**6.4**

The coefficient of variation (%CV) shows the reproducibility of the assay for measuring one antibody activity (sample or control) relative to another antibody activity (Sensitivity Control). Variation increases in the threshold region; for this reason, consider running additional tests for borderline samples.

Sensitivity

The KRV antigen coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate antiKRV IgG from background (non-antibody) signal with rat serum samples diluted 1:100.

Specificity

Purified recombinant protein (E.coli) of the Kilham rat virus major capsid protein (VP2, full length ~65kda,>95%) is used to coat the microwells; thus the assay is specific for antibodies directed to KRV VP2 protein. The Anti-rat IgG HRP conjugate reacts specifically with rat IgG class antibodies that bind to the KRV antigen on the plate. IgA, IgM and IgE antibody would not be measured above background signals.

Precautions

Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Limitations

The assay detects and quantifies IgG antibodies directed the major capsid protein VP2. It may be possible for an animal to have KRV infection without producing antibodies specific to VP2.

Anti-KRV antibody levels of an infected animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer evelopment.